Note

In Vitro Mutagenicity Tests on Capsicum Pepper, Shallot and Nutmeg Oleoresins

Ahmad DAMHOERI,[a] Akiyoshi HOSONO,[b,c] Takatoshi ITOH,[b,d] and Akira MATSUYAMA[b]

AP4 Project, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University, Bogor, Indonesia

Received September 3, 1984

In tropical Asia, various spices have been commonly used as appetizers, food preservatives and traditional medicines. These spices are generally considered as safe because of their long historical and widespread use. Buchanan *et al.*[1] reported that neither nutmeg oleoresin nor chili pepper oleoresin was found to be mutagenic in a *Salmonella*/mammalian microsome mutagenicity assay employing his^- tester strains. On the other hand, they are known to contain different kinds of biologically active and cytotoxic components, and their carcinogenic or mutagenic effects have been described by several authors.[2~6] Considering the still insufficient information on the mutagenic activities of spices, it may be important to examine the mutagenicity *in vitro* of spices using bacterial mutants and to obtain basic data required for the assessment of thier safety with special reference to their chronic effects in cancer induction[7~9] and enhancement of human aging,[10] both of which may involve the process of mutagenesis.

In this laboratory, the mutagenicity of some spices harvested in Indonesia, *i.e.* red pepper (*Capsicum annuum* Linn.), bird pepper (*Capsicum frutescens* Linn.), shallot (*Allium ascalonicum* Linn.), and nutmeg fruit (*Myristica fragrans* Houtt.), were tested. Oleoresins of these spices were prepared by extraction with ethanol. Two streptomycin-dependent (SM^d) strains, SD1018 and SD7823, were isolated from *S. typhimurium* TA100 and TA98, respectively, according to the method of Kada *et al.*,[11] and used in spot tests and plate incorporation tests. For plate incorporation tests, two methods were employed, *i.e.* the SA method in which a 1-ml sample solution is mixed with 9 ml of molten B2 broth agar (10 g Kyokuto beef extract, 10 g Daigo-Eiyo polypeptone, 5 g NaCl and 15 g Difco agar per liter, pH 7.0), and the PI method in which a 0.1-ml sample solution is incorporated into 2 ml of top agar.[12] No metabolic activation of the samples was performed throughout this study.

In spot tests, all oleoresins were found to be mutagenic for both SM^d strains, although those from shallot and nutmeg fruits were not mutagenic for TA98, TA100 and *E. coli* WP2 try^-hcr^-. In plate incorporation tests, all samples except the oleoresins from dried and stored nutmeg seed kernels showed obvious dose-response effects for the mutagenicity in SD1018 and SD7823 more or less. Revertants per gram (RPG) of raw samples, from which the oleoresins were prepared, calculated from the linear parts of the dose-response curves are listed in Table I. With the SA method, RPG values were in the order of 10 for shallot, around 10^2 for capsicum peppers and in the order of 10^3 to 10^4 for nutmeg fruit components, the highest value being for mace. Higher RPG values for red pepper (10^3) were obtained by the PI method as compared with the SA method. It should also noted that the oleoresin of nutmeg kernels prepared from raw seeds with

TABLE I. REVERTANTS PER PLATE PER GRAM SAMPLE[a]

Sample	Test method	Tester strain	
		SD1018	SD7823
Red pepper[b]	SA	1.4×10^2	—
Red pepper[b]	SA	7.3×10	—
Red pepper[c]	SA	8.1×10	—
Red pepper[b]	PI	5.9×10^3	7.9×10^3
Bird pepper[b]	SA	1.0×10^2	—
Bird pepper[c]	SA	3.5×10	6.3×10
Shallot[b]	SA	2.6×10	—
Shallot[c]	SA	2.5×10	—
Nutmeg fruit			
Fruit flesh[b]	SA	1.3×10^3	1.1×10^3
Mace[b]	SA	2.9×10^4	4.6×10^4
Raw kernel[b]	SA	7.9×10^2	1.1×10^3
Raw kernel[b]	SA	—	2.1×10^3
AF2[d]	SA	7.0×10^{10}	8.5×10^{10}
AF2[d]	PI	3.4×10^{11}	4.8×10^{11}

[a] Raw material, from which the oleoresin was prepared.
[b] Autoclaved.
[c] Filtered through a membrane filter.
[d] 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, diagnostic mutagen.

[a] On leave from: *Syiah Kuala University, Banda Aceh, Indonesia.*
[b] *Japan International Cooperation Agency.*
[c] Present address: *Faculty of Agriculture, Shinshu University, Ina, Nagano 399-45, Japan.*
[d] On leave from: *Faculty of Agriculture, Tohoku University, Sendai, Japan.*

light brownish or semi-red mace showed a dose-response mutagenic effect, while that from dried and stored seeds did not, although both of them showed a considerable bactericidal effect. The mutagenic effects of red pepper and shallot were unaffected by the different sterilization methods used for samples, autoclaving at 121°C and filtration through a membrane filter, suggesting that the mutagenic factors in these spices are thermostable. On extraction of red pepper oleoresin with hexane, its major mutagenic and bactericidal factors were found to remain in the residues. Capsaicin, a major pungent principle of capsicum pepper, was negative for SD1018 and SD7823 in spot tests. This may suggest that capsaicin is not involved in capsicum pepper mutagenesis. In contrast, myristicin was positive for both SM^d strains in mutagenicity spot tests. This principal essential oil of nutmeg fruits appears to be responsible for their mutagenicity. Further chemical studies on mutagenic factors involved in these spices would be required. Examination of the antimutagenic material against capsicum pepper mutagenesis was performed with young leaves of the "kemang" tree (*Mangifera caesia* Jack ex Wall.). The "kemang" tree is a native to west Java and its young leaves are locally used to prepare a traditional vegetable salad. The same amount of kemang leaf extracts (13.1 mg dry matter/ml) was added to capsicum pepper oleoresins at different concentrations (up to 7.7 mg dry matter of red pepper and 13.0 mg dry matter of bird pepper). In the plate test (SA method) using SD1018, the water extracts of young kemang leaves were found to exhibit an antimutagenic effect on the mutagenesis of capsicum peppers.

In this study, the lower but definite mutagenic activities of some tropical spices were confirmed. It would be worthwhile to examine the possible chronic effects of the long-term intake of spices commonly used in tropical areas, since such chronic effects as cancer induction and human aging might be linked with the mutagenesis process.

Acknowledgments. The authors thank Dr. T. Kada of the National Institute of Genetics, Japan, for his helpful advice and the kind supply of strains and reagents. They are also indebted to Professor B. N. Ames and his associates at California University, U.S.A., for the strains and references, and Dr. K. Honma of the Hokkaido National Agricultural Experiment Station, Japan, for his discussion on the terminology of spice samples. They are grateful to Mr. Y. Tokizawa of Nomura Trading Co., Ltd., Jakarta, and Takasago Perfumery Co., Ltd., Japan, for reagents. The work was supported by a grant from the Japan International Cooperation Agency.

REFERENCES

1) R. L. Buchanan, S. Goldstein and J. D. Budroe, *J. Food Sci.*, **47**, 330 (1981).
2) C. Hoch-Ligetti, *Acta Unio Intern. Contra Le Cancrum*, **7**, 606 (1951).
3) J. M. Concon, T. W. Swerczek and D. S. Newburg, "Antinutrients and Natural Toxicants in Foods," ed. by R. L. Ory, Food & Nutrition Press, Inc., Westport, Connect., U.S.A., 1981, p. 359.
4) H. Nakamura and T. Yamamoto, *Mutat. Res.*, **103**, 119 (1982).
5) M. Ungsurungsie, O. Suthienkul and C. Paovalo, *Fd. Chem. Toxic.*, **20**, 527 (1982).
6) M. Nagao and T. Sugimura, "Dietary Influences on Cancer: Traditional and Modern," ed. by R. Schoental and T. A. Connors, CRC Press, Inc., Florida, U.S.A., 1982, p. 201.
7) B. N. Ames, W. E. Durston, E. Yamasaki and F. D. Lee, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 2281 (1973).
8) J. McCann, E. Choi, E. Yamasaki and B. N. Ames, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 5135 (1975).
9) T. Kawachi, T. Yahagi, T. Kada, Y. Tajima, M. Ishidate, M. Sasaki and T. Sugiyama, "Molecular and Cellular Aspects of Carcinogen Screening Tests," ed. by R. Montesano, H. Bartsch and L. Tomatis, IARC Scientific Publications No. 27, Lyon, 1980, p. 323.
10) T. Sugimura, Abstracts of Papers, 3rd Intern. Conf. on Environmental Mutagens, 1981, p. 7.
11) T. Kada, K. Aoki and T. Sugimura, *Environ. Mutagenesis*, **5**, 9 (1983).
12) D. M. Maron and B. N. Ames, *Mutat. Res.*, **113**, 173 (1983).